Autoantibodies in Autism Spectrum Disorders (ASD)

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ABSTRACT: Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders defined behaviorally by abnormalities in social, verbal, and nonverbal communication. The etiologies of ASD are unknown, likely to be the result of a variety of numerous genetic, neurological, environmental, and immunological interactions that lead to a general behavioral phenotype defined as ASD. This review will focus on the various immune system anomalies, in particular, autoantibodies, which have been reported in subjects with ASD. In addition, we will discuss recent studies performed by our group concerning the presence of autoantibodies directed against neural antigens, which are observed in patients with ASD.

KEYWORDS: autism; ASD; immunity; autoantibodies; immune system; brain

INTRODUCTION

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders defined behaviorally by abnormalities in social, verbal, and nonverbal communication. They include Asperger's syndrome, autism, child disintegrative disorder, and pervasive developmental disorder, not otherwise specified (PDD–NOS).[1] Stereotypic and restricted behaviors and/or interests are often found in patients with ASD. Patients are diagnosed typically before the age of 36 months, males at a rate four times that of females.[2] The current prevalence is estimated at 1:150 in the total population.[3] There are likely to be

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numerous etiologies for ASD, commonly resulting in a spectrum of disorders with a general behavioral phenotype. Environmental factors suspected of playing a role in the pathogenesis of ASD include pre- and post-natal viral/bacterial infection, metabolic disorders, and perinatal hypoxia.[4] This review will focus on the various changes noted in the immune system of subjects with ASD, in particular the presence of autoantibodies directed against neural antigens, described by both our laboratory as well as others.

GENETICS

ASD has been associated with approximately 15 genes, and is thought to be the product of multiple weak gene interactions. When a broad definition of ASD is taken into account, the concordance rate for monozygotic twins is approximately 60–92%, while in dizygotic twins, this number falls to between 0% and 10%.[5] Familial risk is estimated at 5–10 times higher than the general population, while the sibling rate is 2–6%.[6] In addition, using DNA microarray, it was discovered that in lymphoblastoid cell lines from monozygotic twins discordant in the severity of autism, the most differential gene expression profiles were in genes important to the function, maturation, and structure of the nervous system.[7] Moreover, genes involved in the patterning and growth of the central nervous system (CNS) and its components as well as genes involved in biochemical pathways have been proposed to be involved in the development and maintenance of ASD.[8,9] Taken together, these results suggest that there is a genetic component in the etiology of ASD. Notably, of the genes analyzed in ASD, a number of immune system-related genes have been linked to this disorder. These include the null allele of the C4B gene, a complement component, as well as the extended HLA haplotype B44-S30-DR4, and more recently, A2.[10–12] Recently, Campbell and colleagues discovered that a certain allele resulted in a twofold decrease in the promoter activity of the MET gene and modification in binding of specific transcription factor complexes.[13] This allele was shown to have a genetic association in families of subjects with ASD. MET signaling is not only involved in neocortical and cerebellar development but also immune system functioning and gastrointestinal development and repair, findings all associated with symptoms found in subsets of individuals with ASD. Overall, the genes associated with ASD to date demonstrate an association between the CNS and immune systems and are key in unraveling the etiology of ASD. Future studies must involve a clearly defined subject population with a comparable set of behavioral phenotypes to better draw conclusions regarding the target genes in ASD.

NEUROANATOMICAL OBSERVATIONS IN ASD

The most consistent neuroanatomical alterations observed in patients with ASD involve the cerebellum and the limbic system, though other brain regions

have been observed to be abnormal, including the cerebral cortex, corpus collosum, brain stem, and the basal ganglia.[14–17] In the limbic system, small cell size along with increased cell packing density have been consistently observed in patients with ASD compared with controls.[14] In the cerebellum, the number of purkinje cells have been found to be reduced in patients with ASD.[14] Altered brain growth has also been observed in patients with ASD compared with control groups. The pattern of growth observed is an early, increased rate of growth followed by a reduced growth rate, when compared with controls.[18] These anatomical studies have been examined through the use of brain volume and head circumference measurement, magnetic resonance imaging, and postmortem brain study.[15,18,19] Such observations suggest a long-term, widespread alteration in the development of the CNS, resulting in permanent anatomical changes likely to reflect or be the genesis of functional alterations.

IMMUNE SYSTEM OBSERVATIONS IN ASD

Immune findings in various studies regarding patients with ASD are often inconsistent due to many factors, most notably the use of inappropriate control groups and heterogeneous, ill-defined subject groups. The interpretation of results may be further complicated due to comorbidities, such as mental retardation, epilepsy, sleep disorders, and gastrointestinal symptoms often found in ASD. Various immune system components and mediators including cytokine and immunoglobulin levels, cellular numbers and responsiveness, monocytes/macrophages, and natural killer cells have all been investigated in ASD.[20–24] Despite the lack of consensus, it is widely agreed that a subset of patients with ASD demonstrate abnormal or dysregulated immunity. Furthermore, it has been proposed that autoimmunity may play a role in the pathogenesis and/or maintenance of ASD in some patients. The presence of antibodies to "self" tissue, or autoantibodies, to various proteins has been observed in a subset of patients with ASD (see TABLE 1).

The connection between autoimmunity and ASD was first recognized by Money *et al*.[25] Children with ASD were found to be more likely to have a family member with an autoimmune disease than normal controls. In 1982, Weizman *et al*. reported the presence of a cell-mediated response to brain tissue using the macrophage migration inhibition factor test in 13 of 17 patients with ASD.[26] In 1990, Warren *et al*. observed that 6 of 11 mothers of children with autism had antibodies that were reactive to lymphocytes of their autistic child.[27] These studies demonstrate that the neuroimmune connection may be of primary importance in a subset of subjects with ASD and warrants further investigation in relation to this spectrum of disorders.

Recently, Zimmerman and colleagues[67] observed specific patterns of plasma reactivity to fetal rat brain protein by immunoblotting. Antibodies from mothers

TABLE 1. Autoantibodies reported in Autism spectrum disorders (ASD)

Study	Antibody directed toward	Studies supporting results	Studies in conflict with results
Singh 1993[38]	Myelin basic protein (MBP)	Connolly 2005,[65] Singh 1998[66]	Silva 2004,[44] Zimmerman 2006[67]
Connolly 2005[65]	Brain-derived neurotrophic factor (BDNF) Endothelial cells (EC) Myelin basic protein	None	None
Evers 2002[41]	Heat shock protein 90 (HSP90)	None	None
Vodjani 2004[42]	Gliadin and cerebellar peptides		
Todd and Ciaranello 1985[35]	Serotonin receptor	Singh 1997 (inhibition assay)[37]	Cook 1993,[68] Yuwiler 1992[36]
Connolly 1999[40]	Brain endothelial cells and nuclei	Todd 1988[69]	None
Singh 1997*[43]	Neuron-axon filament protein (NAFP)	None	None
	Glial fibrillary acidic protein (GFAP)		Zimmerman 2006[67]
Singh and Rivas 2004**[39]	Caudate nucleus	Singer 2006[28]	None
	Cerebral cortex		
	Cerebellum	Vodjani 2004[42]	
Vodjani 2002[70]	Myelin basic protein (MBP) Myelin-associated glycoprotein (MAG) Gandlioside Sulfatide α,β crystallin	Connolly 2005,[65] Singh 1993[38]	Silva 2004,[42] Zimmerman 2006[67]

Continued.

TABLE 1. Continued

Study	Antibody directed toward	Studies supporting results	Studies in conflict with results
	Chondroitin sulfate Myelin oligodendrocyte protein Neuron-axon filament protein (NAFP) Tubulin		
Singh 1998***[66]	Myelin basic protein (MBP) Neuron-axon filament protein (NAFP)		
Silva 2004[44]	Unknown ~20 kDa protein		Singh 1993,[38] 1998[66]
Hollander 1999[45]	B lymphocyte antigen increased in patients with Sydenham's Chorea, some obsessive-compulsive patients, Tourette's syndrome patients with repetitive behaviors (D8/17)	None	None

*study used bovine spinal cord as target, plasma used at dilution at 1:50.

** sera used at dilution of 1:25.

***controls used up to 50 years of age (15 adults).

HC = healthy controls; NNI = nonneurological illness; LKSV = Landau–Kleffner Syndrome Variant; HHV-6 = human herpes virus 6; MR = mental retardation; DS = Down's syndrome.

with children with ASD differentially recognized fetal rat brain proteins when compared with plasma from mothers of normal children. Furthermore, children with ASD displayed a pattern separate from their siblings and from typically developing control children, although there was no clear delineation of patterns between children with ASD and children with other neurodevelopmental disorders. The presence of such antibodies possibly denotes a previous exposure, perhaps the result of injury or abnormal development, to certain brain antigens at an increased rate in subjects with ASD compared with typically developing controls. In another study, Singer *et al*. investigated the reactivity of serum autoantibodies to various areas of human brain in sera of children with ASD, siblings of children with ASD, and healthy controls by Western blot and ELISA.[28] Children with ASD had greater reactivity than controls to basal ganglia and frontal lobe at 100 kDa molecular weight. The intensity of reactivity in cingulate gyrus and cerebellum deep nuclei at 73 kDa was greater in children with ASD than in controls. Reactivity was also found to be higher in the siblings of patients with ASD.

Vargas *et al*. recently reported findings of an ongoing immune cytokine activation in the postmortem brains of patients with ASD.[29] Areas affected included the cerebral cortex as well as the cerebellum. Both microglia and astroglia were found to be activated, based on cell appearance and morphology. In addition, glial fibrillary acidic protein (GFAP) staining and altered cytokine profiles were present in brain tissue as well as in cerebrospinal fluid (CSF). Lymphocytes and antibody were found to be absent from the brain tissue, but an accumulation of perivascular macrophages and monocytes were noted, indicative of a possible predominating innate immune activation. The absence of lymphocytic infiltration and antibody deposition may have been due to the small patient numbers (11), which may not be inclusive of the full autistic spectrum. In addition, subsets of patients with immune system dysfunction, represented by autoantibodies, may have been inadvertently omitted. Of note, many of these specimens were from older subjects and thus, these findings could be reflective of downstream changes. The role of maternal immunity during gestation has also been investigated with respect to autism. Dalton *et al*. injected serum from a mother of three: a typically developing child, a child with autism, and a child with a severe language impairment into gestating mice.[30] The offspring of the injected mice demonstrated behavioral changes as well as antibody deposition on purkinje cells and other neurons, in contrast to the offspring of gestating mice injected with sera from mothers of typically developing children. This study suggests the presence of a factor present in the sera of mothers of children with ASD, which is able to alter neurodevelopment and cause behavioral changes. Additionally, Shi and colleagues used a mouse model to demonstrate that a maternal inflammatory response during gestation was sufficient for the generation of behavioral alterations in the offspring including changes in exploratory behavior, sensorimotor coordination, and gating as well as in social behavior.[31] More recently, Croen *et al*. reported no

link between autoimmune disease in the mother within 2 years of gestation and the development of ASD in the offspring. However, mothers diagnosed with asthma or allergy during the second trimester were twice as likely to have a child with ASD than mothers without asthma or allergy.[32]

The effect of the maternal immune response on the developing immune and central nervous systems, as well as prenatal toxicant exposure, has been studied extensively in various psychiatric disorders, including autism and schizophrenia through the use of animal models.[33,34] A study by Meyer *et al*. showed environmental exposures at two critical periods of fetal development resulted in differential effects on future behavioral modifications, neuropathology, and fetal brain cytokine responses.[33] The effects of environmental agents on the immune system were suggested to be dependent on the period of exposure, leading to disturbed postnatal immune functions in genetically susceptible individuals. This research supports the hypothesis that early immune/nervous system disturbances are capable of permanently altering either/both systems.

Various antibodies to self-proteins have been reported in patients with ASD in the past.[35–45] However, it must be emphasized that these autoantibodies have not been associated with pathology, are also found in diseases other than ASD, and are not present in all subjects with ASD. It is not abnormal to detect autoantibodies in normal healthy controls, although elevated titers have usually been associated with pathogenic states of disease, possibly representative of ongoing immune activation. Factors acting in concert with autoantibodies, such as genetics and environmental, may be at play in such a heterogeneous spectrum of disorders.

CURRENT RESEARCH

To explore the possibility of autoantibodies to a conformational epitope or a protein of low abundance in the brain, our lab performed immunohistochemistry studies using brain slices from the nonhuman primate cynomolgus monkey (*Macca fascicularis*). Sera were tested from patients with ASD ($n = 30$), typically developing controls ($n = 25$), subjects with developmental delay ($n = 11$), and siblings of patients with ASD ($n = 18$; both typically developing and with other disorders). Observations thus far have been restricted to the cerebellum. Various levels of reactivity were observed across all groups. Of importance, preliminary results have shown 20% of patients with ASD were observed to have intense staining of what has been proposed to be the golgi cell of the cerebellum, compared with 0% of typically developing controls and 0% of subjects with developmental delay (FIG. 1). These autoantibodies have not been shown to be pathogenic, though they may help delineate which period(s) during neurodevelopment may have been impacted in genetically susceptible individuals with ASD. As the multiple processes involved in

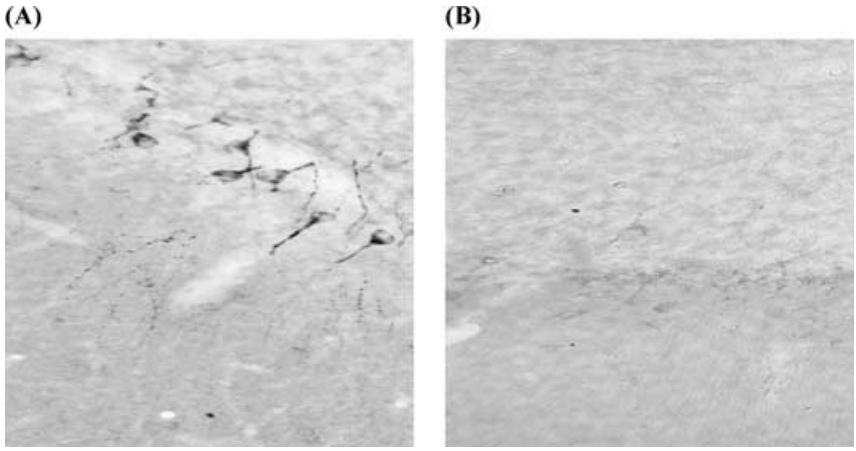

FIGURE 1. Immunohistochemistry of monkey brain cerebellar sections stained with plasma of a patient with ASD (*left*) and from a typically developing control (*right*). Scale bar, 10 μm.

neurodevelopment have been thoroughly outlined, the time of injury/alteration may guide researchers to the disturbed neurodevelopment stages, outlining the specific processes that may occur in some patients with ASD.[46]

To be capable of a pathogenic effect, the neural-specific antibodies would first have to enter the brain. Elegant studies performed by Diamond and colleagues have shown that with blood–brain barrier abrogation, achieved with the use of epinephrine and lipopolysaccharide, anti-DNA autoantibodies associated with systemic lupus erythromatosus (SLE) were able to cause changes in emotional behavior and memory via excitotoxic death caused by cross-reactivity with NMDA (N-methyl d-aspartate) receptors in an animal model.[47,48] Furthermore, additional research has demonstrated that antibodies directed against DNA and other nuclear proteins are capable of entering living cells, binding their intracellular targets, and causing apoptotic death.[49–54]

NEUROIMMUNE CROSSTALK

Several studies have investigated the interdependent relationship between the central nervous and immune systems. Beginning early in development, the relationship between the immune and nervous systems is exceedingly complex, continuing into adulthood mediated mainly through the hypothalamus-pituitary-adrenal (HPA) axis.[55–57] Immune system factors, such as major histocompatibility complex I, cytokines, and chemokines are important in many stages of neurodevelopment and CNS plasticity, functioning, and maintenance. Several proteins associated with the nervous system, such as neuropeptides, have a broad range of effects on the development of the immune system and

its function (suppression as well as activation), including the innervation of immune system-associated organs, such as the lymph nodes and spleen.[58–63] A carefully established equilibrium and timing of the previously mentioned parameters is vital for normal immune and CNS functioning. Changes incurred during development could cause alterations that are lifelong, such as alterations in receptor distribution and/or number in both systems as well as modifications in neuropeptide, cytokine, hormone, or neurotransmitter release during and following neurodevelopment. However, outside of cytokine induction, autoreactive lymphocytes are an essential element in the generation of autoimmunity. Studies by Schwartz and Cohen have shown that in the nervous system, autoreactive T lymphocytes specific for neural antigens were capable of causing disease as well as conferring neuroprotection by promoting repair through the induction of growth factors.[64] The authors propose that the actions of these autoreactive cells are in fact also necessary for protection following tissue damage. It is possible that disruption of this balance, such as in the dysregulated immune system noted in patients with ASD, a shift toward autoimmunity may occur.

CONCLUSION

It is important to remember that ASD are a large, heterogeneous group of disorders resulting from a number of genetic, environmental, neurological, and immunological factors. Also important is to reiterate that the mere presence of autoantibodies is not abnormal and can be found in normal, healthy controls. However, elevated levels, such as those seen in patients with ASD, may be representative of a continuous cycle of immune activation and antibody production, potentially resulting in the generation of pathogenic autoantibodies. Alternatively, these autoantibodies may be an epiphenomenon, possibly corresponding to a period in neurodevelopment that may have been altered. Future research must employ extensive group classification to correlate such findings with a behavioral phenotype. Exploration into neuroimmune crosstalk, particularly during development, will be an essential factor in deciphering the complexity of ASD.

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